Exhibit 32

ARTICLE

Perineal Powder Use and Risk of Ovarian Cancer

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Background	Case-control studies have reported an increased risk of ovarian cancer among talc users; however, the only cohort study to date found no association except for an increase in serous invasive ovarian cancers. The purpose of this analysis was to assess perineal powder use and risk of ovarian cancer prospectively in the Women's Health Initiative Observational Study cohort.
Methods	Perineal powder use was assessed at baseline by self-report regarding application to genitals, sanitary napkins, or diaphragms and duration of use. The primary outcome was self-reported ovarian cancer centrally adjudicated by physicians. Cox proportional hazard regression was used to estimate risk, adjusting for covariates, including person-time until diagnosis of ovarian cancer (n = 429), death, loss to follow-up, or September 17, 2012. All statistical tests were two-sided.
Results	Among 61 576 postmenopausal women, followed for a mean of 12.4 years without a history of cancer or bilateral oophorectomy, 52.6% reported ever using perineal powder. Ever use of perineal powder (hazard ratio [HR]$_{adj}$ = 1.06, 95% confidence interval [CI] = 0.87 to 1.28) was not associated with risk of ovarian cancer compared with never use. Individually, ever use of powder on the genitals (HR$_{adj}$ = 1.12, 95% CI = 0.92 to 1.36), sanitary napkins (HR$_{adj}$ = 0.95, 95% CI = 0.76 to 1.20), or diaphragms (HR$_{adj}$ = 0.92, 95% CI = 0.68 to 1.23) was not associated with risk of ovarian cancer compared with never use, nor were there associations with increasing durations of use. Estimates did not differ when stratified by age or tubal ligation status.
Conclusion	Based on our results, perineal powder use does not appear to influence ovarian cancer risk.

In 2013, it is estimated that there will be 22 240 new cases of ovarian cancer and 14 030 ovarian cancer deaths in the United States (US) alone (1). Since the 1960s, there has been speculation that the use of perineal powder is associated with ovarian cancer. In 2006, the International Agency for Research on Cancer (IARC) reviewed studies examining perineal powder use and ovarian cancer and classified talc as a possible carcinogen (2,3). The proportion of US women ever using talc powder on the perineum was estimated in 2001 to be approximately 40% (4), whereas 52% reported ever use of perineal powder in 1993–1998 within the Women's Health Initiative (WHI) (5).

The primary proposed mechanism linking perineal powder use to ovarian cancer is an inflammatory response (6). Talc particulates from perineal application have been shown to migrate to the ovaries (6), disrupting the surface ovarian epithelial tissue leading to entrapment of the talc particles within inclusion cysts (7). Furthermore, tubal ligation and/or hysterectomy, which would eliminate the pathway of talc particulates to the ovaries, are associated with reduced ovarian cancer risk (6).

A meta-analysis examining the risk of ovarian cancer among ever perineal powder users vs non-users showed odds ratios (ORs)

of 1.40 (95% confidence interval [CI] = 1.29 to 1.52) for population-based case-control, 1.12 (95% CI = 0.92 to 1.36) for hospital based case-control, and 1.35 (95% CI = 1.26 to 1.46) for all case-control studies (2). More recently, a large pooled analysis found that ever use of perineal powder increased epithelial ovarian cancer risk by 24% compared with non-use (OR = 1.24, 95% CI = 1.15 to 1.33) (8). Increased risk was associated with invasive serous, endometrioid, clear cell, and borderline serous subtypes of epithelial ovarian cancer (8). However, when looking at the lifetime number of applications of perineal powder, there was no statistically significant trend for increasing applications, attributed to difficulty in recalling details of frequency and duration of perineal powder use (8).

To date there has only been one prospective study conducted examining perineal powder use and risk of ovarian cancer (9). In the Nurses' Health Study (NHS) cohort, no overall association was found between ever use of perineal powder and epithelial ovarian cancer (relative risk [RR] = 1.09, 95% CI = 0.86 to 1.37) or serous ovarian cancers (RR = 1.26, 95% CI = 0.94 to 1.69) (9). However, there was a 40% (95% CI = 1.02 to 1.91) increase in risk for serous

invasive ovarian cancer with ever perineal powder use, which comprises 86% of serous ovarian cancers in this cohort (9).

Limitations of recall bias and misclassification make it difficult to determine the true relationship between perineal powder (10), a commonly used cosmetic product, and ovarian cancer, a disease with poor survival and few known modifiable risk factors. The prior prospective cohort study, which should not be affected by recall bias, had no information on duration of use limiting interpretation. Here we expand on the available evidence by assessing perineal powder use and risk of ovarian cancer in the Women's Health Initiative Observational Study (WHI-OS). The WHI-OS is a large cohort that collected information on several application areas of perineal powder use and their respective durations of use.

Methods

Study Population

The WHI-OS enrolled 93 676 women from 40 clinical centers across the United States from 1993 to 1998 (11). Women were eligible if they were aged 50 to 79 at enrollment, postmenopausal, and planned to reside in the area for at least three years (11). Women were excluded from the WHI-OS if they were participating in another clinical trial, unlikely to survive three years due to medical conditions, or had conditions that would interfere with study participation (11). Participants completed annual mailed questionnaires to update information on risk factors and outcomes, including ovarian cancer (11). Written informed consent was obtained from participants, and all clinical centers were approved by their respective institutional review boards (11). The current analysis was approved by the University of Massachusetts, Amherst Human Subjects Review Committee.

For this analysis, participants were additionally excluded if they reported a bilateral oophorectomy or an unknown number of ovaries at baseline (n = 20 960), a history of any cancer at baseline except nonmelanoma skin cancer (n = 10 622), or were missing exposure or follow up information (n = 516). After applying the exclusion criteria, 61 576 participants with 429 adjudicated incident ovarian cancer cases remained.

Exposure Ascertainment

Perineal powder use was assessed via self-report at baseline. Participants were asked, "Have you ever used powder on your private parts (genital areas)?" Those who responded yes further indicated the duration of use with the following possible responses: less than 1 year, 1–4 years, 5–9 years, 10–19 years, or 20 or more years. For persons that reported ever use of a diaphragm, participants were asked, "Did you ever use powder on your diaphragm?" and those who responded yes further indicated duration. The third category evaluated was "Did you ever use powder on a sanitary napkin or pad?" with those responding yes also reporting duration. Each area of application variable was assessed dichotomously and the duration of use, collapsed into fewer categories because of small numbers, was assessed categorically as never, 9 years or less, or 10 or more years. A combined ever perineal powder variable and duration variable for any powder use was created; where ever use was defined as report of ever use of any of the three application categories, never was report of never use for all three categories,

and duration was the maximum duration reported of any single area of application, because we could not exclude the possibility that applications were concurrent. Lastly, all possible combinations of the three application areas were assessed.

Outcome Ascertainment

Ovarian cancer cases were initially self-reported by participants in the WHI-OS on annual questionnaires. Medical records, including hospital discharge summaries and pathology reports, were requested for each self-reported case and adjudicated by a physician at the local Clinical Center and then centrally by the WHI's Clinical Coordinating Center (11).

Covariate Ascertainment

Potential covariates considered included age, race, education, alcohol servings per week, smoking status, metabolic equivalent (MET) hours per week of recreational physical activity, Body Mass Index (BMI), and self-reported family history of ovarian or breast cancer. Reproductive factors considered were age at menarche, age at menopause, age at first birth, age at last birth, parity, breastfeeding duration, history of tubal ligation, history of hysterectomy, history of irregular cycles, history of endometriosis, duration of oral contraceptive use, and duration of postmenopausal hormone use. All covariates were from baseline and were not updated.

Statistical Analysis

To estimate the association between perineal powder use and ovarian cancer, proportional hazard regression models were used. Participants contributed person-time until diagnosis of ovarian cancer, death, loss to follow-up, or September 17, 2012, whichever came first. Participants with other cancers were still considered at risk for ovarian cancer and were not censored at the time of other cancer diagnoses. Information on incident oophorectomy during follow-up was not available and thus participants were not censored in this analysis. The proportional hazards assumption was tested using weighted Schoenfeld residuals.

Covariates were included in the adjusted model according to purposeful selection, where covariates with Wald P values of .25 or less in age-adjusted models were entered into an initial multivariable model and then each covariate was subsequently tested individually via likelihood ratio tests in order of decreasing Wald P values. Variables that had P values of .10 or less during the backwards elimination were kept in the model until a parsimonious model was obtained. Additional variables shown in previous literature (8,9) but not statistically significant in our population were also included in the final multivariable model. Lastly, family history of breast cancer and personal history of endometriosis did not change estimates and were not included in the final multivariable model.

Models fitted included the following independent variables: 1) combined ever perineal powder use, 2) ever powder use by application area (ie, applied to genitals, applied to diaphragm, or applied to sanitary napkins), 3) duration of use by application area, and 4) application area combinations (ie, genital only, diaphragm only, sanitary napkin only, genital and sanitary napkin, genital and diaphragm, diaphragm and sanitary napkin, and all three areas of application). For duration models, test for trend was used to evaluate linear trends across duration categories by modeling the

categories as a continuous variable in the multivariable regression models.

Because powder particles may not reach the ovaries due to tubal ligation and because previous studies have shown a stronger association between powder use and ovarian cancer in women without tubal ligation (4), we separately examined women without tubal ligation. We also stratified by age at baseline, because older women may have had more potential for exposure to talc contaminated with asbestos. Additionally, associations by ovarian cancer histological subtype were evaluated. All analyses were performed using Stata v.12.1 (StataCorp, College Station, TX) and two-sided P values of .05 or less were considered statistically significant.

Results

The average age of the participants at baseline was 63.3 years. Participants were followed for a mean of 12.4 years; never powder users were followed for a mean of 12.2 years (range = 0.12 to 17.9 years) and ever powder users were followed for a mean of 12.6 years (range = 0.03 to 18.0). The majority of the participants were white (83.7%), had less than a college degree (56.1%), and were overweight/obese (57.2%). Approximately half (52.6%) of the population reported ever use of perineal powder. Ever powder users were heavier (27.5 kg/m^2 vs 26.5 kg/m^2, P < .0001) and were more likely to have used oral contraceptives (44% vs 36%, P < .0001) and/or diaphragms (50.8% vs 37.3 %, P < .0001) than never users (Table 1).

Use of powder on the genitals was associated with a 12% increase in the multivariable-adjusted hazard ratio of ovarian cancer (HR_{adj} = 1.12, 95% CI = 0.92 to 1.36), though this was not statistically significant (Table 2). Use of powder on sanitary napkins (HR_{adj} = 0.95, 95% CI = 0.76 to 1.20) or diaphragms (HR_{adj} = 0.92, 95% CI = 0.68 to 1.23) also was not associated with risk. Duration of powder use on the genitals, sanitary napkins, or on the diaphragm was not associated with ovarian cancer; P_{trend} for years of use: .67, .69, and .67 respectively. Combined ever powder use from any of the three application areas did not show an association with ovarian cancer risk (HR_{adj} = 1.06, 95% CI = 0.87 to 1.28). For combined duration of use, which was the longest duration of use among the three areas of application, there was no evidence of an association with risk of ovarian cancer [P_{trend} for years of use: .77]. Use of powder on genitals, the most common application area, for 20 or more years was not associated with increased risk of ovarian cancer compared with never users (HR_{adj} = 1.10, 95% CI = 0.82 to 1.48). In a sensitivity analysis, invasive serous ovarian cancer risk was not increased (HR_{adj} = 0.96, 95% CI = 0.65 to 1.41), even in women reporting durations of use greater than 10 years.

There was no evidence of an association between perineal powder use and ovarian cancer risk by category of application (Table 3). Combined ever powder use was not associated with individual subtypes of ovarian cancer (Table 4). The multivariable-adjusted hazard ratio for serous ovarian cancer was 1.16 (95% CI = 0.88 to 1.53). Additionally, duration of combined ever powder use was also not shown to be associated with any subtype of ovarian cancer (results not shown).

The associations of combined ever powder use and ovarian cancer did not statistically differ by tubal ligation status (results not shown). When stratified by age group at baseline, hazard estimates also did not statistically differ ($P_{interaction}$ = .37); HR_{adj} for younger than

Table 1. Characteristics of postmenopausal women according to perineal powder use status (n = 61 285): Women's Health Initiative Observational Study, 1993–2012

Characteristic, n (%)	Never perineal powder use n = 29 066	Ever perineal powder use n = 32 219
Race		
White	24 006 (82.6)	27 336 (84.8)
Nonwhite	4991 (17.2)	4811 (14.9)
Body mass index category, kg/m²		
<25.0	13 056 (44.9)	12 461 (38.7)
25.0–29.9	9734 (33.5)	10 799 (33.5)
30.0 +	5935 (20.4)	8571 (26.6)
Smoking status		
Never	15 347 (52.8)	15 621 (48.5)
Past	11 481 (39.5)	14 339 (44.5)
Current	1912 (6.6)	1881 (5.8)
Duration of oral contraceptive use, y		
Never	17 877 (61.5)	17 954 (55.7)
<5	6241 (21.5)	7858 (24.4)
5 to <10	2528 (8.7)	3270 (10.2)
10 to <15	1650 (5.7)	2125 (6.6)
15+	760 (2.6)	1005 (3.1)
Diaphragm use	10 826 (37.3)	16 353 (50.8)
Tubal ligation	4929 (17.0)	5901 (18.3)
Hysterectomy	6878 (23.7)	8285 (25.7)
Family history of ovarian cancer	606 (2.1)	660 (2.1)
Parity		
0	3687 (12.7)	3769 (11.7)
1–2	9773 (33.6)	11 585 (36.0)
3–4	11 101 (38.2)	12 609 (39.1)
5+	4365 (15.0)	4098 (12.7)
Age at last birth, y		
Never had term pregnancy	6219 (21.4)	6260 (19.4)
< 20	210 (0.7)	324 (1.0)
20–29	9143 (31.5)	11 480 (35.6)
30+	13 011 (44.8)	13 668 (42.4)
Duration of postmenopausal hormone use, y		
Never	13 381 (46.0)	13 880 (43.1)
<5	6498 (22.4)	7546 (23.4)
5 to <10	3783 (13.0)	4567 (14.2)
10 to <15	2688 (9.3)	3128 (9.7)
15+	2716 (9.3)	3097 (9.6)

50 to 59 years = 1.29, 95% CI = 0.91 to 1.82; HR_{adj} for those 60 to 69 years = 0.94, 95% CI = 0.70 to 1.26; and HR_{adj} for those 70 to 79 years = 1.01, 95% CI = 0.68 to 1.48. When restricted to only whites or to those who had never used oral contraceptives, results were again unchanged.

Discussion

In this large prospective study, ever perineal powder use was not associated with ovarian cancer risk, nor was it associated with ovarian cancer when assessed by area of application, duration of use, or ovarian cancer subtype. While many case-control studies have shown an approximately 24–40% increase in risk of ovarian cancer (2,8) for powder users, we did not find evidence of this association in our large, prospective analysis.

The meta-analysis of 20 case-control studies by Langseth and colleagues found a 35% increase in the odds of epithelial ovarian

Table 2. Age and multivariable-adjusted hazard ratios of ovarian cancer by area of perineal powder application (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR (95% CI)	P_{trend} †	Multivariable HR* (95% CI)	P_{trend} †
Powder use on genitals						
Never	247	457 855	1.0 (referent)	.63	1.0 (referent)	.67
Ever‡	181	304 867	1.13 (0.93 to 1.37)		1.12 (0.92 to 1.36)	
Less than 9 years	112	173 118	1.24 (0.99 to 1.55)		1.23 (0.98 to 1.54)	
10 or more years	68	129 647	0.98 (0.75 to 1.29)		0.98 (0.75 to 1.29)	
Powder use on sanitary napkins						
Never	336	590 351	1.0 (referent)	.70	1.0 (referent)	.69
Ever‡	93	172 712	0.96 (0.76 to 1.21)		0.95 (0.76 to 1.20)	
Less than 9 years	62	114 305	0.98 (0.75 to 1.28)		0.96 (0.73 to 1.26)	
10 or more years	30	56 174	0.93 (0.64 to 1.35)		0.95 (0.65 to 1.37)	
Powder use on diaphragm						
Never	373	661 239	1.0 (referent)	.78	1.0 (referent)	.67
Ever‡	52	97 714	0.94 (0.70 to 1.25)		0.92 (0.68 to 1.23)	
Less than 9 years	35	67 468	0.93 (0.66 to 1.32)		0.91 (0.64 to 1.30)	
10 or more years	17	29 202	0.99 (0.61 to 1.60)		0.95 (0.58 to 1.56)	
Combined ever powder use§						
Never	197	361 583	1.0 (referent)	.67	1.0 (referent)	.77
Ever‡	232	404 983	1.07 (0.89 to 1.30)		1.06 (0.87 to 1.28)	
Less than 9 years	135	228 931	1.12 (0.90 to 1.39)		1.09 (0.88 to 1.36)	
10 or more years	97	173 307	1.03 (0.81 to 1.31)		1.02 (0.80 to 1.30)	

* Adjusted for: Age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m² (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children, missing).

† Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models; P_{trend} was estimated by modeling categories as continuous. All statistical tests were two-sided.

‡ Person-years may not add up; duration information was missing for some.

§ Combined ever powder use is the longest duration of use among the applications to genitals, sanitary napkins, and diaphragms.

Table 3. Age and multivariable-adjusted hazard ratios for ovarian cancer by combined categories of powder use (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR* (95% CI)	Multivariable HR* (95% CI)
Powder Type Used				
No powder	193	355 523	1.0 (referent)	1.0 (referent)
Only genital powder	96	158 130	1.14 (0.90 to 1.46)	1.13 (0.88 to 1.45)
Only diaphragm powder	19	42 367	0.82 (0.51 to 1.32)	0.80 (0.50 to 1.29)
Only sanitary napkin powder	28	50 051	1.04 (0.70 to 1.54)	1.01 (0.68 to 1.50)
Genital and sanitary napkin powder	55	96 173	1.09 (0.80 to 1.47)	1.08 (0.80 to 1.46)
Genital and diaphragm powder	24	29 858	1.49 (0.98 to 2.28)	1.45 (0.95 to 2.23)
Diaphragm and sanitary napkin powder	4	6858	1.06 (0.40 to 2.86)	1.02 (0.38 to 2.74)
Genital, diaphragm, and sanitary napkin powder	5	18 331	0.51 (0.21 to 1.24)	0.50 (0.21 to 1.22)

* Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models. All statistical tests were two-sided. Multivariable HR adjusted for: age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m² (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children missing).

cancer among ever perineal powder users compared to never-users (2), and the pooled analysis of eight case-control studies by Terry and colleagues found a 24% increase in the same group (8). Langseth and colleagues did not assess dose-response or risk among subtypes of ovarian cancer (2). Terry and colleagues assessed lifetime applications of perineal powder and found no statistically significant trend with increasing lifetime applications (8). This corroborates our results that there was no statistically significant risk with increasing duration

of perineal powder use, though they were able to capture both frequency and duration (8), whereas we only had duration. Terry and colleagues found elevated risks for invasive serous, borderline serous, endometrioid, and clear cell subtypes of ovarian cancer (8), which we did not observe. One potential reason that case-control studies have found slight increases in risk is the potential for an overestimation of the true association due to recall bias, because the participants are aware of their ovarian cancer status when reporting powder

Table 4. Age and multivariable-adjusted hazard ratios for combined ever powder use by subtype of ovarian cancer (n = 61 576): Women's Health Initiative Observational Study, 1993–2012

Variable	No. of cases	Person-years	Age-adjusted HR* (95% CI)	Multivariable HR* (95% CI)
Serous†				
Never	87	355 523	1.0 (referent)	1.0 (referent)
Ever	117	404 983	1.18 (0.89 to 1.56)	1.16 (0.88 to 1.53)
Serous Invasive				
Never	80	355 523	1.0 (referent)	1.0 (referent)
Ever	105	404 983	1.16 (0.87 to 1.55)	1.13 (0.84 to 1.51)
Mucinous				
Never	12	355 523	1.0 (referent)	1.0 (referent)
Ever	13	404 983	0.98 (0.44 to 2.14)	1.03 (0.47 to 2.27)
Endometrioid				
Never	13	355 523	1.0 (referent)	1.0 (referent)
Ever	20	404 983	1.39 (0.69 to 2.79)	1.29 (0.64 to 2.61)
Other				
Never	47	355 523	1.0 (referent)	1.0 (referent)
Ever	54	404 983	1.04 (0.71 to 1.54)	1.04 (0.70 to 1.54)

* Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated in cox proportional hazard regression models. All statistical tests were two-sided. Multivariable HR adjusted for: age (continuous), race (white, nonwhite, missing), oral contraceptive duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), hormone replacement therapy duration in years (never, <5, 5 to <10, 10 to <15, 15+, missing), family history (yes, no, missing), age (y) at last birth (never, <20, 20 to <30, 30+, missing), body mass index in kg/m² (<25.0, 25.0 to <30.0, 30.0+, missing), smoking (never, past, current, missing), tubal ligation (yes, no, missing), and parity (0, 1 to 2, 3 to 4, 5+, children missing).

† Includes borderline cancers.

exposure. The prospective nature of our study would eliminate the potential for recall bias. Additionally, the case-control studies tended to have a younger population than our study, which included both premenopausal and postmenopausal ovarian cancers (2,8), whereas the WHI cohort consisted only of postmenopausal ovarian cancers. Ovarian cancer that occurs prior to menopause may have a different etiology than ovarian cancer occurring afterwards.

We found similar results to that of the NHS, the only other prospective cohort, which had a similar sample size and number of ovarian cancer cases to our study. Ever use of perineal powder did not appear to be associated with ovarian cancer in the NHS (9), similar to our findings. The results of Gertig and colleagues were also null for use on the genitals and for use on sanitary napkins (9). Additionally, neither our study nor the NHS found associations with serous ovarian cancer, endometrioid, or mucinous ovarian cancers, although subgroup sample size may have reduced statistical power to test these associations. In contrast to our results, the study by Gertig and colleagues found a 40% increase in invasive serous ovarian cancer among ever powder users compared with never powder users (9).

Strengths of our study included large sample size with a substantial number of ovarian cancer cases, a prospective cohort design, good case ascertainment, and detailed information on most ovarian cancer risk factors. We also had information on duration of powder use, qualifiers not available in several earlier studies, including the previous cohort study (2,8,9).

One potential limitation of our analyses includes a lack of information regarding oophorectomy after baseline, which would result in the inclusion of some women not at risk for ovarian cancer in the analytical cohort. However, the impact was likely to be minor, as a previous study in the WHI-OS had reported the number of persons with incident bilateral oophorectomies to be less than 250 (out of more than 90 000 participants) during nearly eight years of follow-up (12). While the prospective nature of the study design

eliminates recall bias, it does not eliminate potential for nondifferential misclassification of the exposure. Women still needed to recall past perineal powder use and duration and thus may have trouble recollecting specifics regarding the use of perineal powder, leading to a bias toward the null. Information regarding powder use was not collected after baseline, and there is potential for never users to begin using powder; however, this is unlikely because the women are postmenopausal, reducing need to use perineal powder on diaphragms or sanitary napkins. We also had no specific data regarding the frequency of powder use in our sample. Frequency of use, as well as duration may influence ovarian cancer risk. We may have been comparing long-term infrequent users with short-term frequent users. If we had frequency of use in addition to the duration, we could have looked at intensity of use, which may be more accurate, and shown a dose response relationship. However, Terry and colleagues did not find a dose response relationship either when taking into account frequency and duration (8).

When restricted to women without tubal ligation status, the estimates for the association between combined ever perineal powder use and ovarian cancer were not increased. While some studies have found stronger associations between powder use and ovarian cancer in women that have not undergone a tubal ligation (4), the results from our study did not support this previous finding. The pooled analysis (8) and the NHS cohort (9) also did not find evidence of stronger associations in women without tubal ligations.

While we had information on duration of use, it is unknown during which years the perineal powder was used. Talc powder had potential for asbestos contamination (13) until 1976, when the Cosmetic, Toiletry, and Fragrance Association required all cosmetic talc products to be free of asbestos (14). Therefore, those using powder prior to 1976 may have been potentially exposed to asbestos, a known carcinogen. The pooled analysis and meta-analysis also included case-control studies not within the United States

(2,8), which potentially have different regulations regarding perineal powder and earlier studies that may have been more likely to include exposure to contaminated perineal powder (2). However, risk estimates in more recent studies are similar to earlier studies (2), reducing the likelihood that confounding by asbestos is driving the findings. Additionally, assuming older women in the cohort could have been exposed longer to perineal powder with potential contamination compared with younger women, we did not see statistically significant differences in risk when stratified by age group, further suggesting asbestos contamination is not a likely explanation.

The WHI-OS queried general perineal powder use rather than talc powder use, and we had no specific information regarding the content of talc in products used, which the previous literature reviewed by IARC suggested to be the possible carcinogen of concern (2). However, the NHS cohort and most studies included within the pooled analyses asked about general perineal powder use as well (2,8,9). In summary, perineal powder use did not appear to be associated with ovarian cancer risk in this large sample of postmenopausal women, even with use for long durations.

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Exhibit 33

Research report

Perineal use of talc and risk of ovarian cancer

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ABSTRACT
Ovarian cancer is one of the most common gynaecological neoplasms, especially in industrialised countries. The aetiology of the disease is not well understood, except that inherited mutations in the breast cancer genes BRCA-1 and BRCA-2 account for up to 10% of all cases,[1] and child-bearing, oral contraceptive use and breast-feeding reduce the risk.[2] Some environmental exposures, notably talc and asbestos, have been suspected as ovarian carcinogens.

Talc refers to both mineral talc and industrial products that contain mineral talc. Mineral talc occurs naturally in many regions of the world and is valued for its softness, platyness, and ability to absorb organic matter. Mineral talc occurs natu rally in a platy (flat) form, but may also occur as asbestiform fibres, which describes its physical form and does not imply the presence of asbestos. The purer forms (approximately 90% mineral talc) are used for cosmetic and hygiene products including baby powders and feminine hygiene products. Perineal use of cosmetic talc is a common practice in the United Kingdom, North America, Australia and some other countries. To our knowl edge accurate estimates of prevalence of use of cosmetic talc are not available. However, the use for female hygiene of body powders, baby pow ders, talcum powders and deodorising powder, most of which contain cosmetic talc in varying amounts, has been reported to be as high as 50% in some countries.[3]

From pathological studies it is known that particles and fibres that enter the body can migrate to distant organs. For instance, asbestos fibres have been found in ovaries from women exposed to asbestos.[4 5] Analogously, following perineal appli cation, talc particles can migrate from the vagina to the peritoneal cavity and ovaries.[6] A majority of women experience retrograde menstruation[7]; this suggests a mechanism by which talc particles can travel through the female reproductive tract to the ovaries. Furthermore, epidemiological studies have shown decreased risks of ovarian cancer after tubal ligation and/or hysterectomy, suggesting that removing a pathway by which carcinogenic sub stances can reach the ovaries reduces the risk.[8 9]

The association between talc use in the perineal region and ovarian cancer was investigated in one cohort study,[10] and 20 case control studies.[11 30] In the cohort study, arguably the strongest study because of its partly prospective ascertainment of exposure, there was no association between cosmetic talc use and risk of all subtypes of ovarian cancer combined. The various case control studies provided indications of either a significant excess risk (10 studies) or non significant excess risk or null (10 studies), with odds ratios (ORs) ranging from 1.0 to 3.9. None of the studies reported relative risks below 1.0. The population based case control studies,[11 15 17 20 26 28 30] included studies with 112 824 ovarian cancer cases, and had odds ratios ranging from 1.1 to 3.9 (fig 1). The hospital based case control studies[12 14 18 19 27] included studies with 77 462 cases, and reported odds ratios between 1.0 and 2.5. Pooled odds ratios were calculated by fixed effects model. As shown in figure 1 pooled ORs were 1.40, 1.12 and 1.35 for population based, hospital based and all case control studies com bined, respectively. Some studies[13 14 22 23 26 28] tried to assess exposure response associations, in terms of frequency of use or length of use in years but found no clear trend.

Methodological factors such as recall bias should always be considered in case control studies. It could have been a problem had there been wide spread publicity about the possible association between use of body powder and cancer. The International Agency for Research on Cancer (IARC) working group considers that there has not been widespread public concern about this issue and therefore considers it unlikely that such a bias could explain the consistent findings. Another source of recall bias could result from the fact that women with the cancer tend to remember or over report their use of body powder. The influence of this type of recall bias cannot be ruled out.

Eight of the population based case control stu dies[11 16 22 24 26 28 29] were identified, by the IARC working group as being most informative in terms of size of the studies, whether the studies were population based, participation rates and adjust ments of confounding variables. The selected studies included at least 188 cases and had participation rates ranging from 60% to 75%. Among these eight studies, the prevalence of perineal use of talc based body powder among controls ranged from 16% to 52%. The relative risks of ovarian cancer among body powder users were homogeneous across this set of eight studies, each of which indicated a 30 60% increase in risk. Among the other 12 case control studies, most also reported relative risks of this magnitude or higher.

Information on talc use in infancy is generally insufficient in the case control studies. However, in one study the exposure to baby powder was reported by 42.2% of the cases and 40.5% of the controls.[15] In several of the other studies patients were asked about age at first use of perineal talc, as an indicator for use in infancy or other periods of life.

Only four case control studies[16 23 29 30] and one cohort study[10] provided results by histological type. In four of these studies, in particular the cohort study, there were hints of higher risks of serous tumours related to talc exposure.
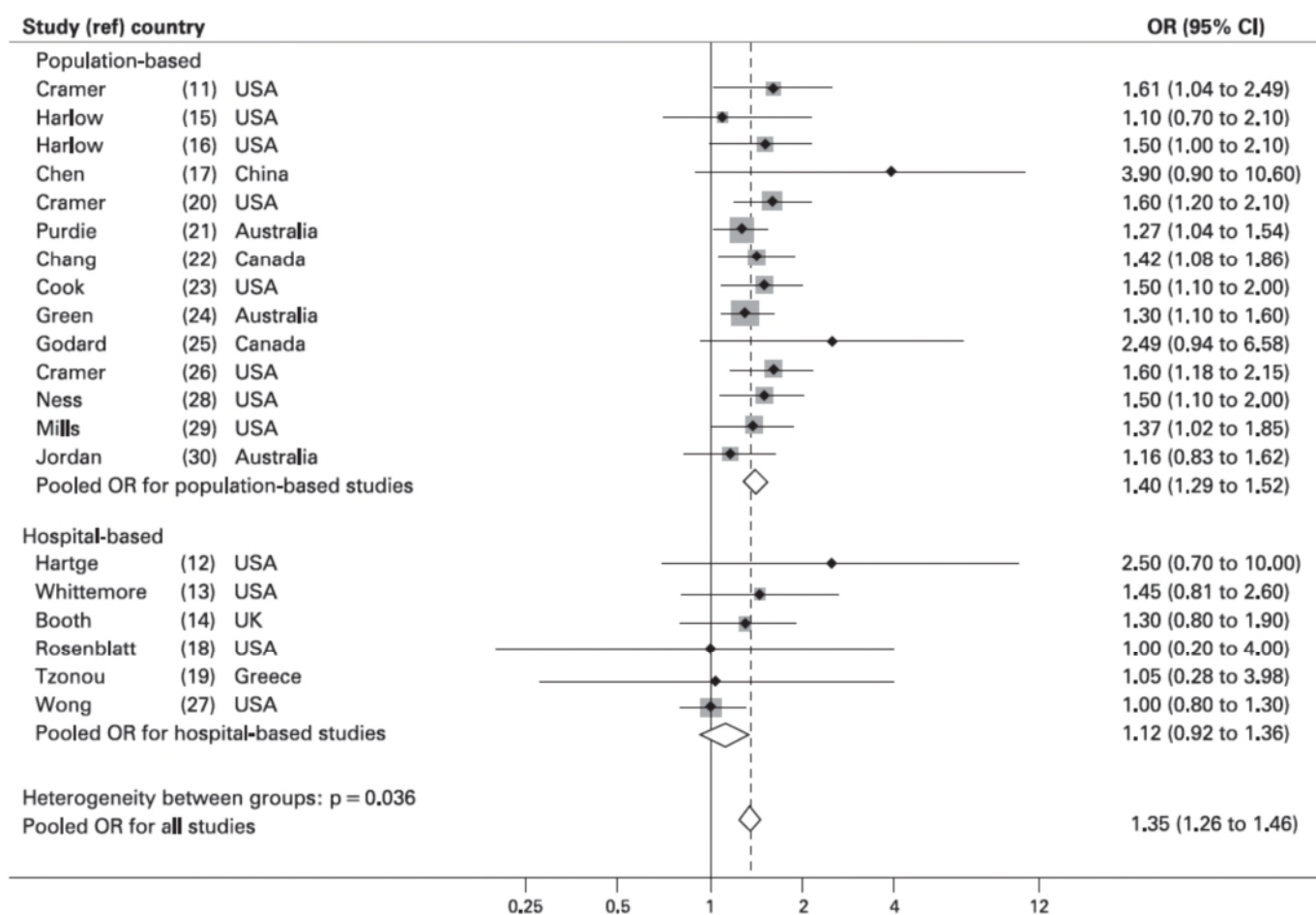
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Study (ref) country			OR (95% CI)
Population-based			
Cramer	(11)	USA	1.61 (1.04 to 2.49)
Harlow	(15)	USA	1.10 (0.70 to 2.10)
Harlow	(16)	USA	1.50 (1.00 to 2.10)
Chen	(17)	China	3.90 (0.90 to 10.60)
Cramer	(20)	USA	1.60 (1.20 to 2.10)
Purdie	(21)	Australia	1.27 (1.04 to 1.54)
Chang	(22)	Canada	1.42 (1.08 to 1.86)
Cook	(23)	USA	1.50 (1.10 to 2.00)
Green	(24)	Australia	1.30 (1.10 to 1.60)
Godard	(25)	Canada	2.49 (0.94 to 6.58)
Cramer	(26)	USA	1.60 (1.18 to 2.15)
Ness	(28)	USA	1.50 (1.10 to 2.00)
Mills	(29)	USA	1.37 (1.02 to 1.85)
Jordan	(30)	Australia	1.16 (0.83 to 1.62)
Pooled OR for population-based studies			1.40 (1.29 to 1.52)
Hospital-based			
Hartge	(12)	USA	2.50 (0.70 to 10.00)
Whittemore	(13)	USA	1.45 (0.81 to 2.60)
Booth	(14)	UK	1.30 (0.80 to 1.90)
Rosenblatt	(18)	USA	1.00 (0.20 to 4.00)
Tzonou	(19)	Greece	1.05 (0.28 to 3.98)
Wong	(27)	USA	1.00 (0.80 to 1.30)
Pooled OR for hospital-based studies			1.12 (0.92 to 1.36)
Heterogeneity between groups: p = 0.036			
Pooled OR for all studies			1.35 (1.26 to 1.46)

0.25   0.5   1   2   4   12

Figure 1 Results from case-control studies contributing data on perineal talc use and ovarian cancer. Results are presented as odds ratios (ORs) and their corresponding confidence intervals (95% CIs) and represented by squares and lines, respectively. Results are separated in 14 population-based and six hospital-based case-control studies. Pooled ORs for all population-based studies combined and all hospital-based studies combined are given. OR pooling by fixed effect models (Mantel-Haenszel method).

Before 1976, talc was to some extent contaminated with asbestos, so that the early studies relating talc to ovarian cancer may have been confounded by the asbestos.[31] However, the association between talc exposure and ovarian cancer is as strong in recent studies,[28 29] as in earlier ones, diminishing the likelihood that all these results are influenced by contamination of talc by asbestos.

To summarise the evidence in favour of an association, a very large number of studies have found that women who used talc experienced excess risks of ovarian cancer; some results were statistically significant and some were not. There was some indication in the cohort study of an increase in serous tumours. The evidence of talc migrating to the ovaries lends credibility to such a possible association. The main epidemiological evidence against the association is the absence of clear exposure response associations in most studies, as well as the absence of an overall excess risk in the cohort study.

On balance, the epidemiological evidence suggests that use of cosmetic talc in the perineal area may be associated with ovarian cancer risk. The mechanism of carcinogenicity may be related to inflammation.[32]

The carcinogenicity of non asbestiform talc was assessed by a monograph working group at IARC in 2006.[33] After considering biases and possible confounding factors, the IARC working group concluded that the epidemiological studies provided limited evidence for the carcinogenicity of perineal use of talc based body powder, and classified this use as possibly carcinogenic to human beings (that is, group 2B).[34]

PROPOSAL: TO RESEARCH COMMUNITY
The current body of experimental and epidemiological evidence is insufficient to establish a causal association between perineal use of talc and ovarian cancer risk. Experimental research is needed to better characterise deposition, retention and clearance of talc to evaluate the ovarian carcinogenicity of talc.

The majority of the epidemiological studies carried out so far have been among American women. It would be instructive to seek evidence in other countries where perineal use of talc has been common.

While there has been some efforts to measure the degree of use, these have mainly been measured simply as the reported years of use. It is possible that the ostensible lack of exposure response trends is the result of crudeness of the exposure metric used. Therefore, it is important that future studies, irrespective of study design, devote some effort to better assessment of exposure. The use of body powders should be assessed both in terms of calendar time and age of the subject. Subjects should be asked about lifetime use, including age at initial use (infancy, childhood, teenager years, adulthood), age at which they stopped using such powders, gaps in the lifetime period of use

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What this study adds

▶ Epidemiological evidence suggests that use of cosmetic talc in the perineal area may be associated with ovarian cancer risk. The IARC has classified this use of talc as possibly carcinogenic to human beings (group 2B).

▶ The mechanism of carcinogenicity may be related to inflammation. This paper focus on the high degree of consistency in the studies accomplished so far, and what should be the focus in future studies.

and frequency and nature of use (daily, during certain seasons of the year, only while menstruating). Another important question is whether the use of body powder was before or after tubal ligation or hysterectomy.

Individuals' answers to questions about use of brand names over time may be unreliable, and therefore, in future studies, investigators should try to ascertain, either from government or industry sources, the composition of the powders used in different time periods by different brand names and, in particular, to ascertain whether the exposure may have included some contamination by asbestos and also whether the exposure was to talc or a non talc product. Statistical analyses should attempt to assess risk separately for the categories of powders: talc containing asbestos, talc not containing asbestos, non talc product. Further, exposure metrics should take into account the age, duration and intensity of exposure. As well as analyses for all ovarian tumours combined, there should, if possible, be analyses by histological subtype and by invasiveness of the tumour.

While it would not be reasonable to envisage establishing a costly long term prospective cohort study just to study this association, any long term cohort study that is being set up to study cancer among women should collect information about talc use if the study is being conducted in a country where such use has been widespread.

In summary, future studies should focus on seeking evidence in talc exposed female populations worldwide, collecting reliable information on age at initial use of body powder, exposure assessments and dose response associations.

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Exhibit 34

Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer

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Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors, such as the use of talcum powder (talc) in the pelvic region and epithelial ovarian cancer. To address this issue we evaluated the potential role of chronic local ovarian inflammation in the development of the major subtypes of epithelial ovarian cancer. Factors potentially linked to ovarian inflammation were examined in an Australia-wide case–control study comprising 1,576 women with invasive and low malignant potential (LMP) ovarian tumors and 1,509 population-based controls. We confirmed a statistically significant increase in ovarian cancer risk associated with use of talc in the pelvic region (adjusted odds ratio 1.17, 95% CI: 1.01–1.36) that was strongest for the serous and endometrioid subtypes although the latter was not statistically significant (adjusted odds ratios 1.21, 95% CI 1.03–1.44 and 1.18, 95% CI 0.81–1.70, respectively). Other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection and mumps) were not associated with risk but, like others, we found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis. Regular use of aspirin and other nonsteroidal anti-inflammatory drugs was inversely associated with risk of LMP mucinous ovarian tumours only. We conclude that on balance chronic inflammation does not play a major role in the development of ovarian cancer.
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Key words: ovarian cancer; chronic inflammation; talcum powder

Chronic inflammation (hereafter referred to as inflammation) was first invoked as a possible mechanism leading to the development of epithelial ovarian cancer to explain observed associations between certain factors, such as use of talcum powder in the perineal region or pelvic inflammatory disease (PID) and risk of ovarian cancer.[1] The major mechanisms thought to underlie ovarian carcinogenesis, namely increased pituitary gonadotropins or incessant ovulation, do not explain such associations.

A link between inflammation and cancer in general has long been recognized. As early as 1863, Virchow noticed the presence of leukocytes in cancer tissues and suggested a possible connection between inflammation and cancer.[2] Since inflammation also represents the process by which the immune system responds to infection or irritation, however, it has been referred to as a 'double-edged sword' with acute (beneficial) inflammation distinguished from the chronic (detrimental) inflammation that may prevent a robust anti-tumour response.[3]

Indeed the most consistent evidence linking inflammation with ovarian cancer comes from the many reports that use of talc in the perineal region increases ovarian cancer risk.[4,5] It has been suggested that the association between talc use and ovarian cancer is strongest for serous tumours when compared to other less common subtypes.[4,6,7] This would be consistent with the histological similarities observed between serous ovarian cancer and mesothelioma, which is known to be caused by asbestos, and the shared

chemical properties of talcum powder and asbestos. Testing various factors that are possibly related to ovarian inflammation in a case–control study, Ness et al.[8] found that perineal talc use and endometriosis, defined as the presence of endometrial tissue outside the uterus and associated with localised inflammation at the site of endometriotic implants, were positively associated with ovarian cancer risk. However, they saw no association with PID, which they had also expected to be associated with increased risk.[8] Extending these epidemiological analyses, McSorley et al.[9] recently found significantly higher circulating C-reactive protein (CRP) levels, a marker of systemic chronic inflammation, among 167 women with incident ovarian cancer risk in a multicentre nested case–control study.

The potential role of ovarian inflammation in the development of ovarian cancer remains an open question. The aim of the current study was to further examine the role of local chronic inflammation in the development of epithelial ovarian cancer overall and by histologic subtype. In addition to talcum powder use, we examined medical conditions that cause inflammation in the pelvic region, including endometriosis and PID, and we also tested the hypothesis that if inflammation causes ovarian cancer then regular use of anti-inflammatory drugs should be inversely associated with this disease.

Material and methods

Study design

The Australian Ovarian Cancer Study is an Australia-wide population-based case–control study of epithelial ovarian cancer. It includes incident cases of invasive and low malignant potential (LMP) ovarian cancer diagnosed in women (aged 18–79 years) between January 2002 and June 2005. A total of 3,553 women were identified with suspected ovarian cancer. Of these, 304 died before contact could be made, physicians refused to give consent to contact 133, usually because they were too sick or unable to give informed consent and 194 women could not be contacted. A further 167 (5%) were excluded on the basis of language difficulties (70), mental incapacity (33) and illness (64). The remaining 2,755 women were invited to participate and, of these, 2,319 (84% of those approached) agreed to take part.

Two researchers independently abstracted information on tumour site, histological subtype and tumour behaviour (invasive vs. LMP) from the diagnostic histopathology reports and discrepancies were resolved by consensus. For a sample of 87 women, the pathology reports and full set of diagnostic slides were reviewed by a gynaecologic pathologist and the agreement with the original abstracted data was more than 97% for tumour site, behaviour and subtype. After histopathology review, 624 women were excluded because they were found to have nonepithelial, nonovarian or benign tumours and 10 because their cancer was first diagnosed before the start of the study period. Of the final 1,685 eligible participants with invasive or LMP cancers of the ovary, peritoneum or fallopian tube, 1,576 (94%) returned a questionnaire and comprised the case population in the current study. Separate analyses were also carried out for the 994 serous, 191 mucinous, 141 endometrioid and 88 clear cell tumours (the remaining 162 tumours were of other epithelial or mixed subtypes).

Potential control participants were identified from the Australian Electoral Roll (all citizens are required by law to enrol). Controls were frequency-matched to the entire case series based on age (5-year groups) and state of residence. In all, 3,600 women were contacted. Of these, 158 were ineligible because of language difficulties ($n = 97$) or illness ($n = 61$) and 16 were unable to be contacted a second time. Of the 3,426 eligible women, 1,612 (47%) agreed to participate and returned a questionnaire. From these women, 6 were excluded because they reported a previous ovarian cancer and 97 because of a previous bilateral oophorectomy resulting in a total of 1,509 controls for study.

Study participants filled in a comprehensive health and lifestyle questionnaire, which included questions about their personal details, physical characteristics, family history, medical and surgical history, lifestyle habits and reproductive factors. To determine use of talcum powder in the perineal region, participants were asked whether they had ever used powder or talc in the genital area or on underwear or sanitary pads/diaphragm. They were asked their age at first use and years of talc use in these areas. Duration of talcum powder use prior to and after hysterectomy/tubal ligation was calculated and in all analyses perineal talc use was defined as use occurring while the reproductive tract was patent (i.e., prior to hysterectomy/tubal ligation for those women who had undergone gynaecological surgery). Information on talc use under the arms or on the chest or abdomen was also collected.

To measure use of nonprescription anti-inflammatory medications, participants were given examples of the type of medication (e.g., aspirin) followed by a list of the common generic and brand names. To quantify the frequency of use, participants were asked how often they had taken various medications over the past 5 years (ranging from never to as much as twice or more per day). The current analyses were restricted to medications known to suppress inflammation namely aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Participants were also asked whether they had ever had any of a number of specific medical conditions and, if so, the ages at which these were diagnosed.

Ethics approval was received from the Human Research Ethics Committees at the Queensland Institute of Medical Research, Peter MacCallum Cancer Centre, University of Melbourne, all participating hospitals and cancer registries.

Statistical analysis

Risk estimates were calculated as odds ratios (OR) with 95% confidence intervals (CI). χ^2-Squared tests were used to test for differences in patient characteristics (e.g., age, level of education). All significance tests were 2-sided and a p-value of less than 0.05 was taken as significant. Unconditional multiple logistic regression models were constructed to simultaneously adjust for confounding factors.

Exposures to factors of interest occurring in the 12 months prior to diagnosis for cases (or 12 months prior to first contact for controls) were excluded because the aetiological influence of very recent exposures on incident ovarian cancer is likely to be minimal and, in cases, recent behaviours may reflect the presence of subclinical disease. All models were adjusted for the categorical variables of age in 10-year groups (<50, 50–59, 60–69, ≥ 70), highest level of education, parity (number of pregnancies >6 months) and duration of contraceptive use (including oral contraceptive pills and contraceptive injections). Analyses of endometriosis and potential symptoms of endometriosis (painful or long periods) were also adjusted for the categorical variable of body mass index (BMI) 1 year prior to diagnosis/recruitment (≤ 24.9, 25–29.9, ≥ 30 kg/m^2). Other potential confounders that were considered for all analyses but not included in the final models since they did not substantially alter risk estimates were: income, family history of ovarian or breast cancer, hysterectomy and/or tubal ligation and smoking.

All analyses were performed using the SAS system V 9.1 (SAS Institute, Cary, NC). Tests for linear trend were performed using the maximum likelihood test with the categorical variable of interest entered as a continuous term.

Results

The final study population included 1,576 women with epithelial ovarian cancer (invasive and LMP) and 1,509 controls. Cases were significantly older than controls (mean age cases = 57.8, mean age controls = 56.42, $p = 0.001$) and were less likely to have continued their education beyond high school (Table I). As expected, cases were significantly more likely to be nulliparous

portion of use in younger women would have been after that date. Significantly elevated risks of ovarian cancer overall and for the serous subtype were seen in women who were 70 years of age or older and also among those who were less than 50 for the serous subtype only. A modest increase in risk was also observed in the 50–59 year group (nonsignificant) however no association was observed in the 60–69 year age group. Similar results were observed when invasive tumours were examined separately (the number of LMP tumours was too small to evaluate the effects by age).

Table III shows no significant association was observed between PID and risk of all subtypes of ovarian cancer combined (OR = 1.15, 95% CI: 0.85–1.57), or for the different histological subtypes. When we examined the association relative to the time elapsed since diagnosis of PID, no association with ovarian cancer risk was observed (data not shown).

A reported history of genital herpes was not associated with risk of all subtypes of ovarian cancer combined (OR = 1.17, 95% CI: 0.73–1.87). However, a significant positive association was seen with risk of serous tumours (OR = 1.65, 95% CI: 1.01–2.69; Table III), with similar nonsignificant increases observed for both invasive (OR = 1.65, 95% CI: 0.98–2.78) and LMP serous tumours (OR = 1.76, 95% CI: 0.71–4.34). For serous tumours, similar increased risks were seen for both more recent (less than 20 years) and long-term (over 20 years) infection (data not shown).

Neither HPV infection, based on self-reported history of abnormal pap smears and/or genital warts, nor a history of mumps after the age of puberty were associated with risk of ovarian cancer overall (Table III). There was also no association with mumps when we considered infection at any age (OR = 0.95, 95% CI: 0.81–1.12). There was however a suggestion that HPV infection was associated with a slightly increased risk of the endometrioid subtype (OR = 1.58, 95% CI: 1.03–2.44). Analyses considering time since the condition was first reported did not alter these results.

We found no significant association between a reported history of endometriosis and ovarian cancer risk overall (OR = 1.31, 95% CI: 0.97–1.78). However statistically significant increased risks were seen for the endometrioid and clear cell subtypes (OR = 1.85, CI: 1.02–3.38 and OR = 2.66, CI: 1.31–5.44, respectively). Because endometriosis may go undiagnosed, we also considered a reported history of potential symptoms of endometriosis (long or painful periods) however neither was associated with ovarian cancer risk (Table III). Similar results were noted when the analysis was restricted to women who had not used hormonal contraceptives. As with other medical conditions, risk estimates did not vary with time elapsed since endometriosis was first reported.

For comparison with inflammation believed to occur in close proximity to the ovaries, medical conditions associated with inflammation at other body sites were also examined (including gall stones, inflammatory bowel disease, diverticulitis, oesophagitis, gastritis and pancreatitis). None of these conditions was associated with ovarian cancer risk (data not shown).

To assess whether regular use of anti-inflammatory medications was inversely associated with ovarian cancer risk, use of aspirin and NSAIDs in the 5 years prior to study recruitment was examined. Any use of aspirin was not associated with ovarian cancer risk for all subtypes combined (OR for any vs. no use = 1.06, 95% CI: 0.92–1.23; Table IV) or for any of the individual subtypes. Ever use of NSAIDs in the last 5 years also had no effect on risk of all subtypes of ovarian cancer (OR = 0.88, 95% CI: 0.76–1.02). However, risk of mucinous tumours was inversely associated with any use of NSAIDs (OR = 0.69, 95% CI: 0.50–0.94) and a further decrease in risk was observed with more frequent use (p-value trend = 0.01). Separate analyses of invasive (n = 44) and LMP (n = 147) mucinous tumours demonstrated that the observed inverse association was driven entirely by LMP tumours (OR for any vs. no use = 0.59, 95% CI: 0.41–0.84, compared to

TABLE III – ASSOCIATION BETWEEN SELF-REPORTED MEDICAL CONDITIONS POTENTIALLY ASSOCIATED WITH INFLAMMATION OF THE OVARIES AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] N (%)	All cases[1] N (%)	All cases (N = 1,576) OR[2] (95% CI)	Serous (N = 994) OR[2] (95% CI)	Mucinous (N = 191) OR[2] (95% CI)	Endometrioid (N = 141) OR[2] (95% CI)	Clear cell (N = 88) OR[2] (95% CI)
PID							
Never	1,406 (94)	1,460 (93)	1.0	1.0	1.0	1.0	1.0
Ever	84 (6)	103 (7)	1.15 (0.85–1.57)	0.96 (0.66–1.38)	1.46 (0.82–2.60)	1.29 (0.66–2.52)	0.87 (0.30–2.49)
Genital herpes							
Never	1,420 (98)	1,425 (97)	1.0	1.0	1.0	1.0	1.0
Ever	35 (2)	42 (3)	1.17 (0.73–1.87)	1.65 (1.01–2.69)	0.40 (0.09–1.71)	0.32 (0.04–2.37)	0.74 (0.10–5.63)
HPV infection							
Never	1,148 (78)	1,197 (81)	1.0	1.0	1.0	1.0	1.0
Ever	317 (22)	273 (19)	0.94 (0.78–1.15)	0.92 (0.74–1.15)	0.98 (0.66–1.45)	1.58 (1.03–2.44)	0.72 (0.36–1.47)
Mumps							
Never	496 (76)	508 (75)	1.0	1.0	1.0	1.0	1.0
Ever (postpubertal)	160 (24)	164 (25)	0.96 (0.73–1.25)	1.06 (0.79–1.42)	0.78 (0.40–1.49)	0.97 (0.50–1.87)	0.81 (0.35–1.92)
Endometriosis[3]							
Never	1,413 (94)	1,431 (92)	1.0	1.0	1.0	1.0	1.0
Ever	87 (6)	124 (8)	1.31 (0.97–1.78)	1.14 (0.80–1.62)	0.89 (0.46–1.75)	1.85 (1.02–3.38)	2.66 (1.31–5.44)
Long periods[3] (>7 days)							
Never/rarely	1,174 (82)	1,173 (82)	1.0	1.0	1.0	1.0	1.0
Often	188 (13)	192 (14)	1.05 (0.83–1.31)	1.05 (0.81–1.36)	0.70 (0.40–1.22)	1.23 (0.71–2.12)	1.26 (0.62–2.53)
Always	75 (5)	62 (4)	0.79 (0.55–1.13)	0.82 (0.55–1.23)	0.78 (0.34–1.78)	0.72 (0.27–1.85)	0.83 (0.24–2.83)
Painful periods[3]							
Never/rarely	760 (52)	711 (49)	1.0	1.0	1.0	1.0	1.0
Sometimes	290 (20)	301 (20)	1.04 (0.85–1.27)	1.04 (0.83–1.31)	0.95 (0.61–1.47)	1.07 (0.65–1.75)	1.13 (0.59–2.15)
Often	404 (28)	452 (31)	1.17 (0.98–1.40)	1.17 (0.96–1.43)	1.12 (0.77–1.64)	1.12 (0.72–1.73)	1.14 (0.65–2.00)

[1]Numbers may not sum to total because of missing data.—[2]Adjusted for age, education, parity and oral contraceptive pill use.—[3]Additionally adjusted for body mass index one year prior to diagnosis.

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TABLE IV – ASSOCIATION BETWEEN ANTI-INFLAMMATORY MEDICATION USE IN THE PAST 5 YEARS AND RISK OF EPITHELIAL OVARIAN CANCER

	Controls[1] N (%)	All cases[1] N (%)	All cases (N = 1,576) OR[2] (95% CI)	Serous (N = 994) OR[2] (95% CI)	Mucinous (N = 191) OR[2] (95% CI)	Endometrioid (N = 141) OR[2] (95% CI)	Clear cell (N = 88) OR[2] (95% CI)
Aspirin							
Never	772 (51)	783 (50)	1.0	1.0	1.0	1.0	1.0
Ever	730 (49)	781 (49)	1.06 (0.92–1.23)	1.06 (0.90–1.25)	0.99 (0.72–1.35)	0.92 (0.64–1.32)	0.92 (0.58–1.45)
≤1/week	612 (41)	650 (41)	1.06 (0.91–1.23)	1.05 (0.88–1.25)	0.98 (0.71–1.36)	0.98 (0.68–1.43)	0.95 (0.59–1.54)
≥2/week	118 (8)	131 (8)	1.06 (0.80–1.41)	1.11 (0.81–1.51)	1.02 (0.52–2.03)	0.56 (0.23–1.34)	0.75 (0.30–1.89)
p-Value (trend)			*0.5*	*0.4*	*0.99*	*0.4*	*0.6*
NSAIDs							
Never	625 (42)	723 (46)	1.0	1.0	1.0	1.0	1.0
Ever	878 (58)	836 (54)	0.88 (0.76–1.02)	0.93 (0.78–1.10)	0.69 (0.50–0.94)	0.76 (0.53–1.09)	0.92 (0.58–1.45)
≤1/week	653 (43)	625 (40)	0.90 (0.76–1.05)	0.94 (0.78–1.12)	0.73 (0.53–1.02)	0.73 (0.50–1.09)	0.97 (0.59–1.60)
≥2/week	225 (15)	211 (14)	0.83 (0.66–1.04)	0.90 (0.70–1.16)	0.51 (0.28–0.93)	0.84 (0.49–1.44)	0.79 (0.39–1.58)
p-Value (trend)			*0.1*	*0.3*	*0.01*	*0.3*	*0.6*

[1]Numbers may not sum to total because of missing data.–[2]Adjusted for age, education, parity and oral contraceptive pill use.

1.17, 95% CI 0.62–2.21 for invasive mucinous tumours). There was also a dose-response relationship for LMP mucinous tumours (OR for 2 or more pills per week *vs.* no use = 0.46, 95% CI: 0.23–0.91, *p*-value trend = 0.01).

Discussion

The hypothesis that chronic inflammation may lead to the development of epithelial ovarian cancer was first proposed to explain how certain factors, such as talc use in the perineal region, may be linked to increased risk of developing ovarian cancer.[1] Testing the inflammation hypothesis in a case–control study, Ness *et al.* found that proinflammatory factors, such as perineal talc use and endometriosis increased ovarian cancer risk, but others such as PID did not significantly increase ovarian cancer risk (separate analyses of individual histological subtypes of ovarian cancer were not presented).[8] Consistent with this hypothesis, McSorley *et al.*[9] recently reported a trend of increasing ovarian cancer risk with increasing levels of CRP, a marker of inflammation. However, given the lack of specificity of CRP and its association with prevalent chronic conditions, such as ischaemic heart disease,[12] it is difficult to rule out confounding as an alternate explanation for these results.[9] Until the present study, no other epidemiological studies appear to have tested the hypothesis that ovarian inflammation is associated with ovarian cancer risk. In the current study, a significantly elevated risk of ovarian cancer overall and of the serous subtype associated with perineal talc use was identified. A nonsignificant increase in risk was also seen for endometrioid tumours. Other factors that could potentially cause ovarian inflammation (such as PID, HPV infection, mumps and endometriosis) were not associated with ovarian cancer risk overall, however there was some evidence of a positive association with some of these factors in the subtype specific analyses. These results in combination with previous studies suggest that chronic inflammation is unlikely to play a major role in the development of ovarian cancer.

Focusing on talc use, we found that any use of perineal talc was associated with a small but significantly increased risk of ovarian cancer overall and specifically amongst the invasive and LMP serous tumours although no clear dose-response with increasing duration of use was identified. This finding is consistent with results of previous studies.[4,6,7,10,13,14]

As expected, ovarian cancer risk was only related to talc use in women with no surgical closure of the fallopian tubes or those who had used talc presurgery, with no association seen for talc use after tubal sterilisation or hysterectomy. Similar observations were made in previous case–control studies of ovarian cancer (all subtypes) with elevated risks observed in women who had not had a tubal ligation[4,14] or those who had used talc presurgery.[13] These former studies together with the current findings support the hypothesis that talc particles are transported to the ovaries *via* unob-

structed fallopian tubes. In contrast, the Nurses' Health study found no increase in risk among women who were perineal talc users but had never had a tubal ligation.[7]

While it has been demonstrated experimentally that talc particles can reach the ovaries in humans and rodents as the result of talc use in the pelvic region,[15–17] ovarian talc particle burden in normal human ovaries is not correlated with reported exposure levels.[17] This suggests that use of only a small amount of talc may be required for some talc to reach the ovaries and increase risk of cancer.

It has been hypothesised that talc is linked to ovarian cancer development through inflammation, however evidence linking an inflammatory response with talc contamination of the ovaries is lacking. Talc-induced inflammation is unlikely to be in the formation of granulomas as these are rarely observed in human ovaries.[18,19] Other likely manifestations of talc-induced inflammation include reduced fibrinolysis, activation of neutrophils and macrophages and increased production of cytokines and growth factors, and these have been suggested to occur in the peritoneum in response to contamination by surgical glove powder.[20] Rigorous investigation of the precise biological response of the ovarian surface epithelium to perineal talc use is needed.

We also sought to determine whether possible contamination of talc with asbestos fibres, which are known to cause inflammation of epithelial tissues, could explain the observed link between perineal talc use and serous ovarian cancer. Voluntary guidelines to prevent asbestos contamination of cosmetic talc were introduced in 1976 and consequently earlier formulations were more likely to contain asbestos fibres.[10,11] Increased risk of serous ovarian cancer was not restricted to perineal talc use in the oldest age groups, who were more likely to have been exposed to asbestos-contaminated talc, but was also observed in the youngest (less than 50 years) and the 50–59 year old age group. Other studies have also reported no increase in risk of all subtypes of ovarian cancer associated with talc use before 1970[13] or before 1975.[14] These findings contrast with 2 other reports of increased risk of serous[7] and all subtypes of epithelial ovarian cancer[10] associated with earlier use of talc.

If inflammation plays a role in the aetiology of ovarian cancer then it would be expected that PID would be associated with increased risk of ovarian cancer. PID was not associated with elevated risk of ovarian tumours in our data, confirming several previous reports of no association with PID in studies of all subtypes of ovarian cancer.[8,21,22] To date there has been only one report of a significant positive association between PID and ovarian cancer.[23] Genital herpes infection was associated with a nonsignificant increased risk of invasive serous cancer in our data, although this observation was based on a small number of exposed cases (*n* = 27). One previous study found no association between genital herpes and ovarian cancer risk (the number of exposed cases was not reported).[8] Latent infection by herpes virus is established

in the nerve root ganglia and it is associated with a variety of initial and recurrent symptoms such as genital ulceration.[24] It is biologically plausible that inflammation associated with genital herpes infection could increase risk of ovarian cancer as Herpes simplex virus type 2 has been detected in the upper genital tract of women with acute PID[25,26] and acute salpingitis.[27] Further studies are needed to confirm this association.

HPV infection (based on reports of abnormal pap smears and/ or genital warts) showed no association with ovarian cancer risk, except for the endometrioid subtype. We hypothesised that HPV infection could potentially cause ovarian inflammation as HPV DNA has been identified in the ovaries of patients with primary ovarian squamous intraepithelial neoplasia[28,29] and in the upper genital tract of patients with cervical squamous carcinoma.[30] In addition, high-risk HPV DNA has been reported in 10% of ovarian epithelial carcinomas.[31] Abnormal pap smears and genital warts are generally associated with HPV genotypes classified as high-risk and low-risk, respectively, in regards to their association with carcinogenic transformation.[32] However, separate analyses also showed no association with ovarian cancer risk.

Mumps infection (either after puberty or at any age) was not associated with ovarian cancer risk. It has been estimated that some 5% of postpubertal mumps cases are associated with clinically apparent oophoritis, which in severe cases could result in infertility caused by nonfunctional ovarian tissue.[33] We were unable to identify these particular cases in the current analysis and therefore further study is needed to examine the association between mumps oophoritis and ovarian cancer.

While endometriosis is a condition associated with localised inflammation, it is also related to changes in hormone levels (increased oestrogen unopposed by progesterone) at the site of endometriotic implants.[34] Despite this, endometriosis or potential symptoms of endometriosis (long or painful periods) were not associated with ovarian cancer risk overall, but there was an increased risk of endometrioid and clear cell subtypes among women who reported a history of endometriosis. This result was anticipated because current epidemiological evidence suggests that endometriosis is most strongly associated with the endometrioid and clear cell subtypes of ovarian cancer.[35,36]

Finally, if inflammation did promote epithelial ovarian cancer development, then it may be reasonably expected that regular use of anti-inflammatory medications would reduce risk. However, no overall association with ovarian cancer risk was observed in the current study. This supports results from 2 recent meta-analyses, which have also not shown that regular use of anti-inflammatory medications (aspirin or other NSAIDs) reduces ovarian cancer risk.[37,38] Of interest however was the apparent inverse association between NSAID use and the mucinous subtype, which was entirely driven by the LMP group. We know from other epidemiological studies that the aetiology of mucinous tumours differs in a number of ways from the other subtypes of ovarian cancer, so NSAID use may be another factor to add to this list. However, this result awaits confirmation by others.

Strengths of our study included its large size (1,576 women with ovarian cancer and 1,509 population-based controls) and Australia-wide coverage. A limitation was the low response rate for controls (47%), which could have resulted in selection bias and possibly led to an over-representation of healthy subjects among the controls. Indeed our hysterectomy rate among controls was ~5% lower than expected, but as there are no obvious links between hysterectomy and inflammation that we have not considered, we do not believe that these small differences would have affected the present results. A healthy control bias would most likely influence the analyses of medical conditions, specifically sexually transmitted infections (STIs). For example, if participating controls were less likely to have had an STI this could bias risk estimates for STIs upwards. While we saw a positive association between herpes infection and ovarian cancer risk, there was no association with other STIs suggesting that our ORs are not systematically biased. Overall, a small number of participants reported STIs and it is possible that STIs were underreported because of possible asymptomatic infection or because of the negative connotations associated with having an STI. It is also possible that controls would be more likely to underreport STIs than cases therefore potentially biasing the risk estimates upwards. Another general limitation was that analyses of medical conditions were based entirely on self-reported medical history and as a result the accuracy of these reports could not be confirmed, although self-reports of these miscellaneous conditions are unlikely to be influenced greatly by case/control status.

In summary, most factors that could potentially cause ovarian inflammation (such as PID, HPV infection, and postpubertal mumps) were not associated with a significant elevation in ovarian cancer risk in our study. In addition, the expected corollary, an inverse association with regular use of anti-inflammatory medications, was not observed. While some subtype-specific associations were observed, these were not strong and showed no coherent pattern of association within or across subtypes, aside from the well-recognised increase in risk of endometrioid and clear cell cancers among women with endometriosis. The elevation in ovarian cancer risk associated with use of talc in the perineal region that we and others have observed has been regarded as the main evidence supporting an inflammatory mechanism in the development of epithelial ovarian cancer. However, experimental evidence that perineal talc use elicits an inflammatory response in the ovaries is lacking and overall we conclude that chronic inflammation does not play a major role in the development of ovarian cancer.

Acknowledgements

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